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(54) Title: METHOD OF TREATING ESTROGEN RECEPTOR POSITIVE CARCINOMA

(57) Abstract: This invention provides a method of treating or inhibiting an estrogen receptor positive carcinoma in a mammal in need thereof, which comprises providing said mammal with an effective amount of a combination of a rapamycin and an antiestrogen.

METHOD OF TREATING ESTROGEN RECEPTOR POSITIVE CARCINOMA

BACKGROUND OF THE INVENTION

This invention relates the use of a combination of a rapamycin and an antiestrogen in the treatment or inhibition of estrogen receptor positive carcinoma, particularly breast and ovarian cancer.

Breast cancer is a leading cause of female cancer deaths in the world. The growth of some human breast cancer cells is under hormonal control. Substantial evidence suggests that estrogen promotes the development of breast cancer. The biological effect of estrogen in the breast is mediated by estrogen receptor (ER), which is a member of a large family of ligand-inducible transcription factors. Upon binding to its receptor, the ligand initiates the dissociation of heat shock proteins from the receptor, receptor dimerization, phosphorylation, and binding to DNA response elements of target genes. After binding to DNA, ER differentially regulates transcription of target genes with or without other transcription factors and coactivators/corepressors. Estrogen action can be partially blocked by antagonists (antiestrogens) which act through ER in a way that is competitive with estrogen but fails to activate genes that promote cell growth. The antiestrogen tamoxifen (Tam) has been the first-line therapy in the treatment and management of breast cancer based on the estrogen responsiveness for stimulation of tumor growth. Unfortunately, the effectiveness of Tam therapy is hampered by its agonist activity in other tissues such as the uterus and side effects like hot flashes. There is a need to develop new antiestrogens or to develop optimal combinations of antiestrogens with other therapeutic agents to achieve better efficacy and reduce side effects.

Rapamycin is a macrocyclic triene antibiotic produced by Streptomyces hygroscopicus, which was found to have antifungal activity, particularly against Candida albicans, both in vitro and in vivo [C. Vezina et al., J. Antibiot. 28, 721 (1975); S.N. Sehgal et al., J. Antibiot. 28, 727 (1975); H. A. Baker et al., J. Antibiot. 31, 539 (1978); U.S. Patent 3,929,992; and U.S. Patent 3,993,749]. Additionally, rapamycin alone (U.S. Patent 4,885,171) or in combination with picibanil (U.S. Patent 4,401,653) has been shown to have antitumor activity.

A rapamycin ester, rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid [disclosed in U.S. Patent 5,362,718], also known as CCI-779, has been shown to have antitumor activity against a variety of tumor cell lines, in *in vivo* animal tumor models, and in Phase I clinical trials. [Gibbons, J., Proc. Am. Assoc. Can. Res. 40: 301 (1999); Geoerger, B., Proc. Am. Assoc. Can. Res. 40: 603 (1999); Alexandre, J., Proc. Am. Assoc. Can. Res. 40: 613 (1999); and Alexandre, J., Clin. Cancer. Res. 5 (November Supp.): Abstr. 7 (1999)].

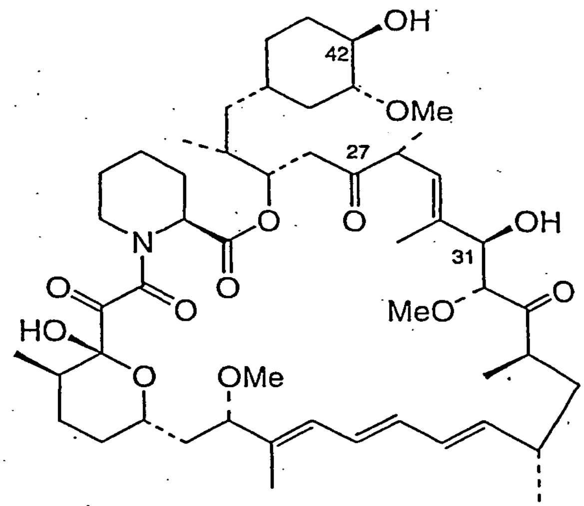
Non-uterotrophic antiestrogens have been reported to have antitumor activity [see, US Patent 5,998,402]. 2-(4-Hydroxy-phenyl)-3-methyl-1-[4-(2-piperdin-1-yl-ethoxy)-benzyl-1H-indol-5-ol, also known as ERA-923, has been reported as being developed for the treatment of estrogen receptor positive metastatic breast cancer. [Gandhi, T., 2000 ASCO Program/Proceedings, Abstract 875, (May 2000)].

DESCRIPTION OF THE INVENTION

This invention provides a method of treating or inhibiting estrogen receptor positive carcinoma in a mammal in need thereof, which comprises providing an effective amount of a combination of a rapamycin and an antiestrogen to said mammal.

This invention also provides a product comprising a rapamycin and an antiestrogen for administration as a combined preparation for simultaneous, separate or sequential use in treating or inhibiting an estrogen receptor positive carcinoma in a mammal.

As defined herein, the term "a rapamycin" defines a class of immunosuppressive compounds which contain the basic rapamycin nucleus (shown below). The rapamycins of this invention include compounds which may be chemically or biologically modified as derivatives of the rapamycin nucleus, while still retaining immunosuppressive properties. Accordingly, the term "a rapamycin" includes esters, ethers, oximes, hydrazones, and hydroxylamines of rapamycin, as well as rapamycins in which functional groups on the rapamycin nucleus have been modified, for example through reduction or oxidation. The term "a rapamycin" also includes pharmaceutically acceptable salts of rapamycins, which are capable of forming such salts, either by virtue of containing an acidic or basic moiety.



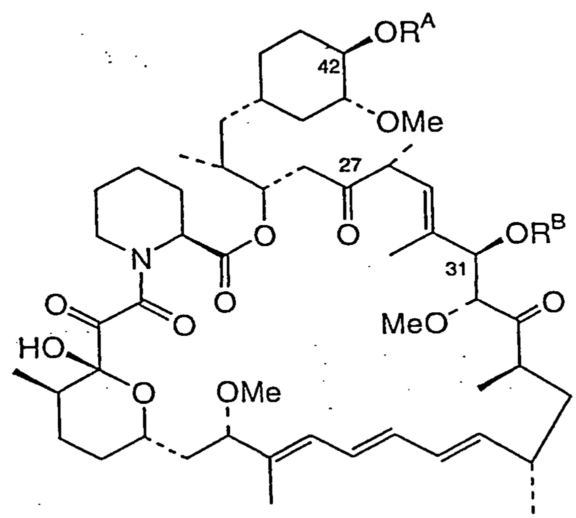
RAPAMYCIN

It is preferred that the esters and ethers of rapamycin are of the hydroxyl groups at the 42- and/or 31-positions of the rapamycin nucleus, esters and ethers of a hydroxyl group at the 27-position (following chemical reduction of the 27-ketone), and that the oximes, hydrazones, and hydroxylamines are of a ketone at the 42-position (following oxidation of the 42-hydroxyl group) and of 27-ketone of the rapamycin nucleus.

Preferred 42- and/or 31-esters and ethers of rapamycin are disclosed in the following patents, which are all hereby incorporated by reference: alkyl esters (U.S. Patent 4,316,885); aminoalkyl esters (U.S. Patent 4,650,803); fluorinated esters (U.S. Patent 5,100,883); amide esters (U.S. Patent 5,118,677); carbamate esters (U.S. Patent 5,118,678); silyl ethers (U.S. Patent 5,120,842); aminoesters (U.S. Patent 5,130,307); acetals (U.S. Patent 5,51,413); aminodiesters (U.S. Patent 5,162,333); sulfonate and sulfate esters (U.S. Patent 5,177,203); esters (U.S. Patent 5,221,670); alkoxyesters (U.S. Patent 5,233,036); O-aryl, -alkyl, -alkenyl, and -alkynyl ethers (U.S. Patent 5,258,389); carbonate esters (U.S. Patent 5,260,300); arylcarbonyl and alkoxycarbonyl carbamates (U.S. Patent 5,262,423); carbamates (U.S. Patent 5,302,584); hydroxyesters (U.S. Patent 5,362,718); hindered esters (U.S. Patent 5,385,908); heterocyclic esters (U.S. Patent 5,385,909); gem-disubstituted esters (U.S. Patent 5,385,910); amino alkanoic esters (U.S. Patent 5,389,639); phosphorylcarbamate esters (U.S. Patent 5,391,730); carbamate esters (U.S. Patent 5,411,967); carbamate esters

(U.S. Patent 5,434,260); amidino carbamate esters (U.S. Patent 5,463,048); carbamate esters (U.S. Patent 5,480,988); carbamate esters (U.S. Patent 5,480,989); carbamate esters (U.S. Patent 5,489,680); hindered N-oxide esters (U.S. Patent 5,491,231); biotin esters (U.S. Patent 5,504,091); O-alkyl ethers (U.S. Patent 5,665,772); and PEG esters of rapamycin (U.S. Patent 5,780,462). The preparation of these esters and ethers are disclosed in the patents listed above.

Accordingly examples of rapamycin compounds include compounds of formula:



wherein R^A and R^B are each selected from hydrogen and ester or ether forming groups as disclosed in any one of the abovementioned US patents.

Preferred 27-esters and ethers of rapamycin are disclosed in U.S. Patent 5,256,790, which is hereby incorporated by reference. The preparation of these esters and ethers are disclosed in the patents listed above.

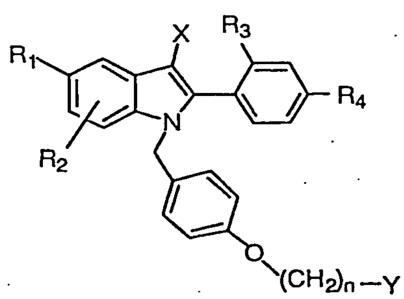
Preferred oximes, hydrazones, and hydroxylamines of rapamycin are disclosed in U.S. Patents 5,373,014, 5,378,836, 5,023,264, and 5,563,145, which are hereby incorporated by reference. The preparation of these oximes, hydrazones, and hydroxylamines are disclosed in the above listed patents. The preparation of 42-oxorapamycin is disclosed in 5,023,263, which is hereby incorporated by reference.

Particularly preferred rapamycins include rapamycin [U.S. Patent 3,929,992], rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid [U.S. Patent 5,362,718], and 42-O-(2-hydroxy)ethyl rapamycin [U.S. Patent 5,665,772].

As used in accordance with this invention, the term antiestrogen is defined as a compound that will blunt or block the effects of an estrogen agonist, such as 17β-estradiol, when administered concommitantly in a test system. The term non-uterotrophic means antiestrogens which typically will not produce clinically significant endometrial proliferation.

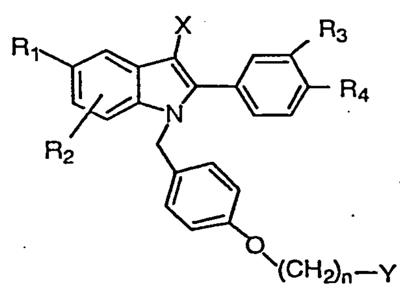
Preferred antiestrogens include compounds such as triphenylene antiestrogens including tamoxifen and 4-hydroxytamoxifen; clomiphene; and non-uterotrophic antiesestrogens, such as those shown below in formulas I and II, raloxifene, droloxifene, idoxifine, nafoxidine, toremifene, TAT-59, levomeloxifene, LY-353381, CP-336156, MDL-103323, EM-800, and ICI-182,780.

Preferred non-uterotrophic antiestrogens include compounds of formulas I or II having the structures



(I)

or



(II)

wherein:

R_1 is H, OH, carboalkoxy of 2-12 carbon atoms, alkoxy of 1-12 carbon atoms, halogen or mono- or poly-fluoroalkoxy of 1-12 carbon atoms;

R_2 is H, OH, carboalkoxy of 2-12 carbon atoms, alkoxy of 1-12 carbon atoms, halogen, mono- or poly-fluoroalkoxy of 1-12 carbon atoms, cyano, alkyl fo 1-6 carbon atoms, or trifluoromethyl, with the proviso that, when R_1 is H, R_2 is not OH.

R_3 and R_4 are each, independently, H, OH, carboalkoxy of 2-12 carbon atoms, alkoxy of 1-12 carbon atoms, halogen, mono- or poly-fluoroalkoxy of 1-12 carbon atoms, or cyano, with the proviso that, when R_1 is H, R_2 is not OH.

X is H, alkyl of 1-6 carbon atoms, cyano, nitro, triflouromethyl, or halogen;

n is 2 or 3;

Y is a saturated, partially saturated or unsaturated 5-7 membered heterocycle containing a nitrogen, which may optionally contain a second heteroatom selected from the group consisting of -O-, -NH- , alkylamine of 1-6 carbon atoms, -N= , and $S(O)_m$;

m is 0-2;

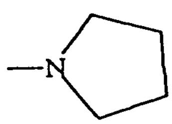
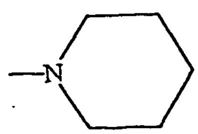
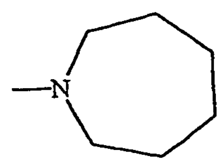
or a pharmaceutically acceptable salt thereof.

Preferred compounds are those in which

R_1 is selected from H, OH or the C_1-C_{12} esters or alkyl ethers thereof, halogen;

R_2, R_3, R_4, R_5, and R_6 are independently selected from H, OH or the C_1-C_{12} esters or alkyl ethers thereof, halogen, cyano, C_1-C_6 alkyl, or trihalomethyl, preferably trifluoromethyl, with the proviso that, when R_1 is H, R_2 is not OH;

X is selected from H, C_1-C_6 alkyl, cyano, nitro, triflouromethyl, halogen;

and Y is  ,  , or 

The preparation of the anti-estrogens of Formulas I and II and pharmaceutically acceptable salts of these compounds are disclosed in U.S. Patent 5,998,402, which is hereby incorporated by reference.

Specifically preferred anti-estrogens of formulas I and II are shown in the Tables below.

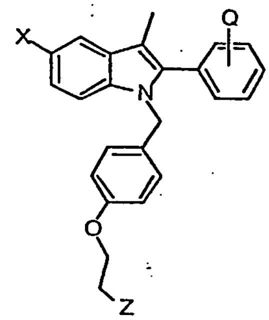


Table 1

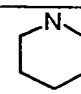
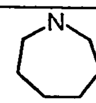
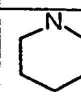
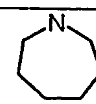
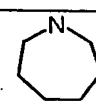
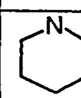
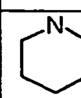
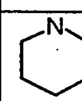
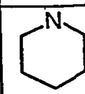
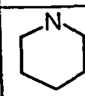
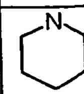
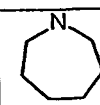
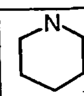
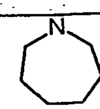
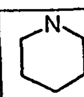
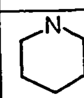
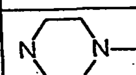
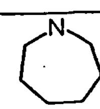
Example No.	X	Q	Z
No. 1	OBn	4'-OEt	
No. 2	OBn	H	
No. 3	OBn	4'-OBn	
No. 4	OBn	4'-OBn	
No. 5	OBn	4'-F	
No. 6	OBn	4'-F	
No. 7	OBn	4'-Cl	
No. 8	OBn	3',4'-OCH_2O-	

Table 1 (Cont'd)

Example No.	X	Q	Z
No. 9	OBn	4'-O-iPr	
No. 10	OBn	4'-CH_3	
No. 11	OBn	3'-OBn	
No. 12	OBn	3'-OBn	
No. 13	OBn	4'-OBn,3'-F	
No. 14	OBn	4'-OBn,3'-F	
No. 15	OBn	3'-OMe	
No. 16	OBn	4'-OCF_3	
No. 17	OBn	4'-OBn	
No. 18	OBn	3'-OMe	

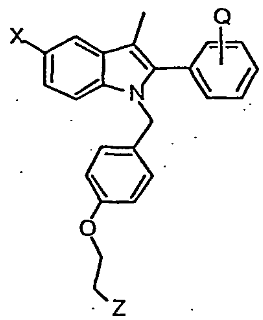


Table 2

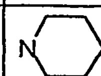
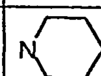
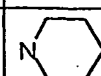
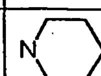
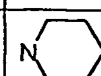
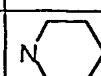
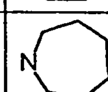
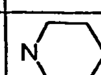
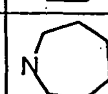
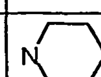
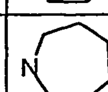
Example No.	X	Q	Z
No. 19	H	H	
No. 20	H	4'-OH	
No. 21	OH	H	
No. 22	OMe	4'-OH	
No. 23	OH	4'-OMe	
No. 24	OMe	4'-OMe	
No. 25	OMe	4'-OMe	
No. 26	OH	4'-OEt	
No. 27	OH	4'-OEt	
No. 28	F	4'-OH	
No. 29	OH	H	

Table 2 (Cont'd)

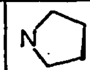
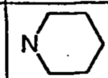
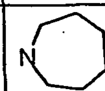
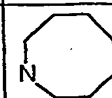
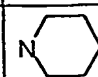
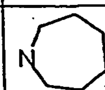
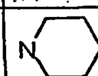
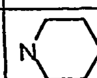
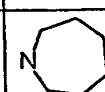
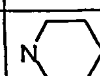
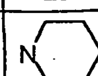
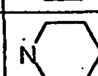
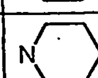
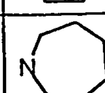
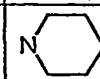
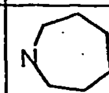
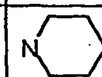
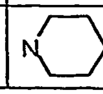
Example No.	X	Q	Z
No. 30	OH	4'-OH	
No. 31	OH	4'-OH	
No. 32	OH	4'-OH	
No. 33	OH	4'-OH	
No. 34	OH	4'-F	
No. 35	OH	4'-F	
No. 36	OH	3'-OMe,4'-OH	
No. 37	OH	3',4'-OCH_2O-	
No. 38	OH	4'-O-iPr	
No. 39	OH	4'-O-iPr	
No. 40	OH	4'-O-Cp	
No. 41	OH	4'-Cl	
No. 42	OH	2',4',-Dimethoxy	
No. 43	OH	3'-OH	
No. 44	OH	3'-OH	

Table 2 (Cont'd)

Example No.	X	Q	Z
No. 45	OH	4'-OH,3'-F	
No. 46	OH	4'-OH, 3'-F	
No. 47	OH	3'-OMe	
No. 48	OH	4'-OCF_3	

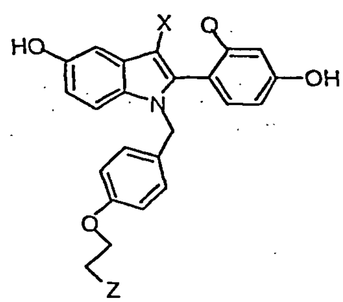


Table 3

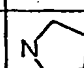
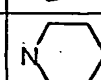
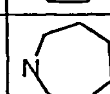
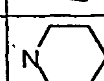
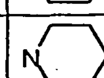
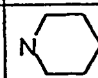
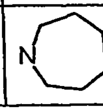
Example No.	X	Q	Z
No. 49	Cl	H	
No. 50	Cl	H	
No. 51	Cl	H	
No. 52	Cl	CH_3	
No. 53	Et	H	

Table 3 (Cont'd)

Example No.	X	Q	Z
No. 54	CN	H	
No. 55	CN	H	

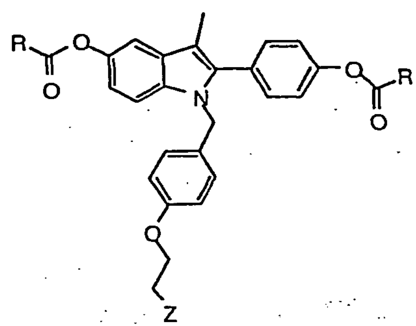
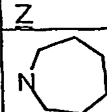
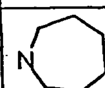
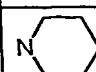


Table 4

Example No.	R	Z
No. 56	Et	
No. 57	t-Bu	
No. 58	t-Bu	

Particularly preferred anti-estrogens of Formulas I or II are those of Examples 31 (2-(4-hydroxy-phenyl)-3-methyl-1-[4-(2-piperdin-1-yl-ethoxy)-benzyl-1H-indol-5-ol) and 32 (1-[4-(2-azepan-1-yl-ethoxy)-benzyl]-2-(4-hydroxy-phenyl)-3-methyl-1H-indol-5-ol) in the tables above.

When applicable, pharmaceutically acceptable salts can be formed from organic and inorganic acids, for example, acetic, propionic, lactic, citric, tartaric, succinic, fumaric, maleic, malonic, mandelic, malic, phthalic, hydrochloric, hydrobromic, phosphoric, nitric, sulfuric, methanesulfonic, napthalenesulfonic, benzenesulfonic, toluenesulfonic, camphorsulfonic, and similarly known acceptable aids when the rapamycin or antiestrogen contains a suitable basic moiety. Salts may also be formed from organic and inorganic bases, such as alkali metal salts (for example, sodium, lithium, or potassium) alkaline earth metal salts, ammonium salts, alkylammonium salts containing 1-6 carbon atoms or dialkylammonium salts containing 1-6 carbon atoms in each alkyl group, and trialkylammonium salts containing 1-6 carbon atoms in each alkyl group, when the rapamycin or antiestrogen contains a suitable acidic moiety.

As used in accordance with this invention, the term "providing," with respect to providing a compound or substance covered by this invention, means either directly administering such a compound or substance, or administering a prodrug, derivative, or analog which will form the equivalent amount of the compound or substance within the body.

The ability of the combination of the rapamycins and antiestrogens of this invention to treat or inhibit estrogen receptor positive carcinoma was confirmed in three standard pharmacological test procedures which measured the ability of the rapamycin antiestrogen combination to inhibit the growth of MCF-7 breast cancer cells and BG-1 ovarian cancer cells, as representative estrogen receptor positive carcinoma. In these test procedures, rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methyl-propionic acid was used as a representative rapamycin, and 2-(4-hydroxy-phenyl)-3-methyl-1-[4-(2-piperdin-1-yl-ethoxy)-benzyl-1H-indol-5-ol, raloxifene, and 4-hydroxy-tamoxifen were used as representative antiestrogens. The following briefly describes the procedures used, and results obtained.

MCF-7 human breast cancer cells were maintained at 37^{0}C in a humidified atmosphere of 5% CO_2 and 95% air in IMEM medium supplemented with 10% fetal bovine serum. BG-1 human ovarian cancer cells were maintained at 37^{0}C in a humidified atmosphere of 5% CO_2 and 95% air in IMEM medium supplemented with 10% fetal bovine serum, 2 ug/ml insulin, and 1% non-essential amino acids. One day before the experiment, cells were plated in 96-well plates at a cell density of 2,500

cells/well in IMEM medium supplemented with 10% fetal bovine serum. The next day, cells were treated with various concentrations of antiestrogen and rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid (referred to as CCI-779 in the tables below). Five or six-days later, cell growth was monitored. MTT solution (20 ul) (0.5 mg/ml) was added to each well and cells were incubated for an additional 4 h. The solution was then removed and 150 ul of DMSO added. The intensity of dark blue color corresponding to number of cells was read at 540 nM with an automated plate reader.

The following tables summarize the results obtained; 2-(4-hydroxy-phenyl)-3-methyl-1-[4-(2-piperdin-1-yl-ethoxy)-benzyl-1H-indol-5-ol is referred to as ERA-923 in the tables below.

Table A. Effect of CCI-779 and ERA-923 on MCF-7 Cells (Percent Inhibition)

CCI-779 / ERA-923	0 nM	5 nM	10 nM	50 nM	100 nM	200 nM	400 nM
0 nM	0	-13.25	-1.54	26.68	37.93	34.99	39.43
0.25 nM	9	13.92	40.37	77.67	84.23	84.64	87.24
0.5 nM	7.59	47.78	70.46	87.07	90.76	89.93	87.42
1 nM	37.56	63.14	80.48	90.95	91.47	91.04	92.6
2 nM	54.65	68.96	78.89	90.89	90.83	92.19	92.71
3 nM	59.39	75.41	84.98	90.71	92.55	92.57	94.17
4 nM	60.04	71.03	83.33	92.48	91.72	93.81	95.77
5 nM	61.12	84.72	87.19	93.6	93.99	95.41	95.34

Table B. Effect of CCI-779 and 4-Hydroxytamoxifen on MCF-7 Cells (Percent Inhibition)

CCI-779 / TAM	0 nM	5 nM	10 nM	50 nM	100 nM	200 nM	400 nM
0 nM	0	-16.48	1.47	14.99	24.63	25.25	33.96
5 nM	20.44	33.31	44.98	73.78	73.72	74.18	75.37
10 nM	22.75	44.37	51.72	75.38	75.07	74.9	77.81
20 nM	39.85	50.36	57.31	74.27	73.72	74.37	76.75
30 nM	41.46	48.39	58.97	70.8	71.72	73.51	76.15
40 nM	39.09	47.82	51.27	71.21	70.35	73.27	75.77
50 nM	47.19	51.47	50.99	70.38	69.74	71.2	73.77
100 nM	36.99	52.68	54.2	69.37	65.81	68.84	73.74

Table C. Effect of CCI-779 and Raloxifene on MCF-7 Cells
(Percent Inhibition)

CCI-779	0 nM	5 nM	10 nM	50 nM	100 nM	200 nM	400 nM
RAL							
0 nM	0	-13.83	-3.3	35.34	38.06	38.08	39.81
0.25 nM	-1.4	-13.31	11.5	50.51	56.51	58.87	64.17
0.5 nM	-5.17	-4.35	28.95	68.66	68.18	75.15	79.38
1 nM	17.3	37.14	50.4	83.52	83.26	88.04	88.85
2 nM	36.78	45.73	65.83	87.68	88.55	91.79	93.91
3 nM	42.4	53.84	70.14	87.8	89.19	89.53	92.86
4 nM	50.49	60.19	75.74	89.19	91.04	91.7	93.26
5 nM	50.5	69.24	78.18	89.73	90.49	93.05	95.09

Table D. Effect of ERA-923 (Alone) on MCF-7 Cells
(Percent Inhibition)

ERA-923	0 nM	0.25 nM	0.5 nM	1 nM	2 nM	3 nM	4 nM
ERA-923							
0 nM	0	6.69	8.17	42.1	67.81	52.23	74.49
0.25 nM	-0.78	35.93	53.61	66.83	74.73	75.24	79.39
0.5 nM	26.09	56.79	63.08	75.09	75.08	76.06	80.92
1 nM	54.91	64.27	68.58	74.24	75.36	77.68	77.2
2 nM	71.82	76.33	73.71	74.07	75.03	76.67	78.2
3 nM	87.65	78.99	81.21	76.56	78.5	81.51	79.28
4 nM	78.72	78.39	79.57	80.45	81.23	83.2	83.28
5 nM	79.25	79.68	81.43	82.97	82.02	82.87	85.92

Table E. Effect of CCI-779 (Alone) on MCF-7 Cells
(Percent Inhibition)

CCI-779	0 nM	5 nM	10 nM	50 nM	100 nM	200 nM	400 nM
CCI-779							
0 nM	0	0.59	4.91	36.55	42.35	45.87	55.78
5 nM	-4.19	13.25	18.78	48.28	54.2	51.41	61.12
10 nM	10.25	18.61	23.5	46.91	52.94	57.1	61.15
50 nM	49.79	49.24	51.18	50.31	50.96	57.24	60.02
100 nM	46.83	50.99	54.81	54.62	55.02	58.97	61.97
200 nM	53.45	58.07	62.43	57.54	62.49	65.48	66.3
400 nM	55.32	64.09	65.21	60.17	66.75	66.84	69.68

Table F. Effect of 4-Hydroxytamoxifen (Alone) on MCF-7 Cells
(Percent Inhibition)

TAM / TAM	0 nM	5 nM	10 nM	50 nM	100 nM	200 nM	400 nM
0 nM	0	12.73	27.87	39.46	46.41	45.75	48.85
5 nM	25.02	37.12	47.71	48.51	52.37	54.54	52.28
10 nM	37.37	49.04	47.11	51.71	50.34	51.06	58.29
20 nM	44.9	50.12	45.86	46.36	47.64	52	56.61
30 nM	42.42	50.39	47.6	42.54	48.22	49.75	57.28
40 nM	46.46	54.19	53.22	48.3	51.79	59.13	59.68
50 nM	45.78	53.75	56.54	50.98	55.29	59.05	67.28
100 nM	49.99	59.23	58.11	58.57	61.34	61.13	64.92

Table G. Effect of CCI-779 and ERA-923 on BG-1 Cells
(Percent Inhibition)

CCI-779 / ERA-923	0 nM	5 nM	10 nM	50 nM	100 nM	200 nM	400 nM
0 nM	0	19.98	22.68	49.76	58.79	55.65	61.94
0.25 nM	14.72	59.39	54.18	57.92	69.12	73.33	77.85
0.5 nM	24.14	66.01	44.73	73.29	82.21	84.25	88.45
1 nM	48.93	74.8	65.78	85.61	94.05	90.99	91.67
2 nM	57.19	92.93	82.02	85.96	90.91	90.31	91.24
3 nM	72.54	94.97	88.57	90.2	93.96	93.91	92
4 nM	80.34	92.13	81.74	90.97	91.56	93.3	94.85
5 nM	75.94	91.99	85.03	86.85	94.3	95.78	95.59

Table H. Effect of CCI-779 and 4-Hydroxytamoxifen on BG-1 Cells
(Percent Inhibition)

CCI-779 / TAM	0 nM	5 nM	10 nM	50 nM	100 nM	200 nM	400 nM
0 nM	0	21.38	24.93	56.31	56.37	60.68	64.93
5 nM	23.99	44.37	41.86	69.61	66.63	71.14	79.24
10 nM	21.39	55.09	59.32	68.95	65.73	72.51	75.3
20 nM	35.05	56.7	60.76	73.68	75.57	75.71	78.67
30 nM	36.43	52.12	52.53	73.61	71.76	73.18	79.34
40 nM	39.51	54.51	54.16	72.6	74.87	75.9	75.12
50 nM	47.73	53.87	61.25	75.35	75.54	80.41	83
100 nM	50	63.03	66.39	76.05	76.66	82.41	83.99

Table I. Effect of CCI-779 and Raloxifene on BG-1 Cells
(Percent Inhibition)

CCI-779 / RAL	0 nM	5 nM	10 nM	50 nM	100 nM	200 nM	400 nM
0 nM	0	8.26	12.46	37.69	42.15	48.36	53.03
0.25 nM	0.74	3.26	18.41	41.02	44.63	43.67	49.32
0.5 nM	-3.32	12.13	25.71	38.32	42.81	50.22	51.64
1 nM	7.79	21.5	33.74	47.59	52.48	56.95	61.41
2 nM	9.85	29.22	40.95	61.14	60.83	62.95	65.37
3 nM	11.5	35.79	44.63	63.98	64.96	68.01	73.02
4 nM	14.49	40.85	48.93	64.55	67.44	72.09	73.88
5 nM	14.04	49.46	48.45	74.98	72	76.32	76.51

An analysis was conducted using a 3-D graphing shareware, MacSynergy II, developed by Prichard and his colleagues (Prichard and Shipman, 1990: 1992) to determine whether the rapamycin plus antiestrogen combination synergistically inhibited estrogen receptor positive carcinoma. Briefly, theoretical additive interactions were calculated from the dose-response curves of each individual drug based on Bliss independence model. The calculated additive surface was then subtracted from the experimental surface to obtain a synergy surface representing % inhibition above the calculated additive value-synergy index. Any peak above the 0% plane suggests synergy. Likewise, any peak below the 0% plane is indicative of antagonism. The results are summarized in the table below for the combinations of rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid plus the antiestrogens shown in the lefthand column.

Compound	MCF-7		BG-1	
	Synergy ($\mu M^2\%$)	Antagonsim ($\mu M^2\%$)	Synergy ($\mu M^2\%$)	Antagonsim ($\mu M^2\%$)
ERA-923	138.0	0.0	25.9	0.0
4-OH Tamoxifen	77.00	0.0	0.3	0.0
Raloxifene	113.0	0.0	40.3	-1.8

The results obtained in these standard pharmacological test procedures showed that the combination of a rapamycin and an antiestrogen synergistically inhibited the growth of estrogen receptor positive carcinoma. For example, the results showed that treatment of MCF-7 breast cancer cells with a combination of ERA-923 and CCI-779,

caused a definite synergistic enhancement of growth inhibition. The growth-inhibitory effect of ERA-923 in the presence of non-inhibitory concentrations of CCI-779 was increased by approximately 10-fold as estimated by the IC_{50}, whereas growth inhibition by CCI-779 in the presence of non-inhibitory concentrations of ERA-923 was increased approximately 40-fold. As shown in Table A, synergy was more pronounced at low concentrations of ERA-923 (0.25 nM-1 nM) in combination with high concentrations of CCI-779 (300-400 nM). The highest degree of synergy of observed was about 60% above additive value.

Similar results were shown with the antiestrogens raloxifene and 4-hydroxy-tamoxifen, in combination with CCI-779. Both raloxifene and 4-hydroxytamoxifen demonstrated synergistic inhibition in the presence of CCI-779. Synergistic inhibition of MCF-7 cells with 4-hydroxytamoxifen in combination with CCI-779 having optimal concentrations ranging from 5 to 20 nM (4-hydroxytamoxifen) and 5 to 400 nM (CCI-779) (Table B). The combination of raloxifene and CCI-779 was also synergistic at a broad range of concentrations of either drug ranging from 0.25-5.0 nM raloxifene and 5.0-400 nM CCI-779 (Table C)

Synergistic inhibition was also demonstrated in estrogen-dependent BG-1 ovarian cancer cells when antiestrogens except 4-hydroxytamoxifen were combined with a rapamycin, such as CCI-779 (Table G, H, I). The failure of 4-hydroxytamoxifen and CCI-779 to demonstrate synergistic inhibition in BG-1 cells is probably associated with 4-hydroxytamoxifen's partial agonist activity in ovary.

In the third standard pharmacological test procedure, mice implanted with MCF-7 tumors were treated with a combination of ERA-923 and CCI-779. The following briefly summarizes the procedure used and results obtained. ERA-923 was dissolved in 1% Tween 80 and 0.9% NaCl Injection USP. Drug was aliquoted into daily doses using 10.0 ml glass bottles and frozen at –20°C until needed. CCI-779 was made fresh using a 5% ETOH, 4.9% Phosal and 0.1% Tween 80 in Sterile Water. 0.2 ml of each drug was given orally starting the day after tumor implantation. ERA 923 was given daily for the duration of the experiment and CCI-779 was given every other day for the first 10 days. The control vehicles were given on the same regime as the drugs. MCF7 cells were cultured in IMEM containing 5% Fetal Bovine Serum with passages varying from 2-20. After trypsinization tumors were resuspended in IMEM with 2% Serum at a 1:1 ratio with Matrigel. 10 million cells were injected subcutaneously into the mammary tissue of

each mouse using a 1.0 ml tuberculin syringe with a 23 ¾ gauge needle. Five to six week old female ovariectomized athymic nu/nu mice (Charles River Labs: Wilmington, MA) weighing from 20.0-23.0 g were used. The animals were housed 5 to a cage in a Microisolator Open Rack System (Lab Products: Maywood, NJ). Each mouse received a 17β-Estradiol pellet (0.72 mg/pellet-60 day release). The pellets were injected with a 10 gauge trochar into the lateral side of the neck between the ear and the shoulder 1-2 weeks prior to tumor injection. Control groups were 15 mice/vehicle whereas the drug groups were 10 mice/drug. Tumors were measured weekly by means of solar calipers (Cole-Parmer Instuments: Vernon Hills, IL) and tumor weights were estimated from tumor diameters by the following formula:

$$\text{Tumor weight (mg)} = \text{tumor length (mm)} \times \text{tumor width (mm)}^2/2.$$

Mice were euthanized 36 days after tumor injection by CO_2 inhalation. The following summarizes the results that were obtained. Nude mice bearing MCF-7 tumors were given ERA-923, CCI-779 or the combination of both drugs. Under these conditions, ERA-923 or CCI-779 had a partial effect (approximately 35% inhibition of growth; growth inhibition = experimental value – 200 (baseline) / control value –200). However, the combination of the drugs inhibited tumor growth approximately 85%. No signs of toxicity were observed with this drug combination. The results are summarized in the table below.

Effect of combination treatment with CCI-779 and ERA-923 on the growth of MCF-7 breast carcinoma in nude mice[1]

Days after tumor implantation	Control treatment[2]	5 mg/kg CCI-779 (PO; q2d days 1-9)[3]	20 mg/kg ERA-923 (PO qd days 1-35)[4]	5 mg/kg CCI-779 plus 20 mg/kg ERA-923[5]
14	495 ± 46	260 ± 18*	480 ± 91	290 ± 22-/*
21	789 ± 83	485 ± 53*	679 ± 127	331 ± 34*/*
28	1111 ± 134	704 ± 63	842 ± 163	398 ± 33*/*
36	1425 ± 179	1123 ± 134	993 ± 197*	498 ± 88*/*

1. Ovariectomized nude mice were given 0.72 mg 60-day slow release 17β-estradiol pellets one week prior to implantation with 1 x10^7 MCF-7 tumor cells near the animal's mammary glands. One day after tumor implantation, oral drug therapy (PO) was given as indicated. Tumor size was measured on the days specified according to previously stated methods (see Discafani et al., 1999. Biochem Pharmacol. 57: 917-925).
2. Control treatment is the combined effect of animals treated with vehicle for CCI-779 (phosal), vehicle for ERA-923 (tween), or vehicle for both drugs. The average tumor sizes of each control group did not significantly vary from each other and therefore were pooled. Values are mean ± standard error. Statistical analysis was done using log transformation of the data followed by ANOVA; pair-wise comparisons were done. The single asterisks refers to statistical significance at p <0.05 compared to the control group. The double asterisk (*/*) or -/* refers to statistical significance,or lack therof, in a pair

wise comparison with CCI-779 or ERA-923 given alone. The values in the CCI-779 plus ERA-923 group were statistically different compared to control at the $p < 0.001$ level.
3. CCI-779 in phosal was given orally every other day beginning on day 1 and terminating on day 9.
4. ERA-923 in tween was given orally every day beginning on day 1.
5. Drugs were given as two separate doses. Dosing was done on the days specified in the groups where the drug was given alone.

Based on the results obtained in the standard pharmacological test procedure described above, the combination of a rapamycin and an antiestrogen are useful in treating or inhibiting estrogen receptor positive carcinoma, particularly estrogen receptor positive breast or ovary carcinima.

It is understood that the effective dosage of the combination of a rapamycin and antiestrogen may vary depending upon the particular compound utilized, the mode of administration, the condition, and severity thereof, of the condition being treated, as well as the various physical factors related to the individual being treated. It is projected that a combination of CCI-779 (as a representative rapamycin) and ERA-923 (as a representative antiestrogen) will be administered once weekly at a projected dosage of 5-500 mg CCI-779 (with a preferred dosgae of 50-200 mg) and 2-500 mg ERA-923 (with a preferred dosage of 25-100 mg). Initial dosages of other rapamycins and antiestrogens can be obtained by comparing the relative potencies with CCI-779 and ERA-923.

As used in this invention, the combination regimen can be given simultaneously or can be given in a staggered regimen, with the rapamycin being given at a different time during the course of chemotherapy than the antiestrogen. This time differential may range from several minutes, hours, days, weeks, or longer between administration of the two agents. Therefore, the term combination does not necessarily mean administered at the same time or as a unitary dose, but that each of the components are administered furing a desired treatment period. For example, in the combination of CCI-779 and ERA-923, it is anticipated that the CCI-779 will be administered parenterally, and the ERA-923 will be administered orally. The combination can be administered daily, weekly, or even once monthly. As typical for chemotherapeutic regimens, a course of chemotherapy may be repeated several weeks later, and may follow the same timeframe for administration of the two agents, or may be modified based on patient response.

Such doses may be administered in any manner useful in directing the active compounds herein to the recipient's bloodstream, including orally, via implants, parenterally (including intravenous, intraperitoneal and subcutaneous injections), rectally, intranasally, vaginally, and transdermally. For the purposes of this disclosure, transdermal administrations are understood to include all administrations across the surface of the body and the inner linings of bodily passages including epithelial and mucosal tissues. Such administrations may be carried out using the present compounds, or pharmaceutically acceptable salts thereof, in lotions, creams, foams, patches, suspensions, solutions, and suppositories (rectal and vaginal).

Oral formulations containing the active compounds of this invention may comprise any conventionally used oral forms, including tablets, capsules, buccal forms, troches, lozenges and oral liquids, suspensions or solutions. Capsules may contain mixtures of the active compound(s) with inert fillers and/or diluents such as the pharmaceutically acceptable starches (e.g. corn, potato or tapioca starch), sugars, artificial sweetening agents, powdered celluloses, such as crystalline and microcrystalline celluloses, flours, gelatins, gums, etc. Useful tablet formulations may be made by conventional compression, wet granulation or dry granulation methods and utilize pharmaceutically acceptable diluents, binding agents, lubricants, disintegrants, surface modifying agents (including surfactants), suspending or stabilizing agents, including, but not limited to, magnesium stearate, stearic acid, talc, sodium lauryl sulfate, microcrystalline cellulose, carboxymethylcellulose calcium, polyvinylpyrrolidone, gelatin, alginic acid, acacia gum, xanthan gum, sodium citrate, complex silicates, calcium carbonate, glycine, dextrin, sucrose, sorbitol, dicalcium phosphate, calcium sulfate, lactose, kaolin, mannitol, sodium chloride, talc, dry starches and powdered sugar. Preferred surface modifying agents include nonionic and anionic surface modifying agents. Representative examples of surface modifying agents include, but are not limited to, poloxamer 188, benzalkonium chloride, calcium stearate, cetostearl alcohol, cetomacrogol emulsifying wax, sorbitan esters, colloidol silicon dioxide, phosphates, sodium dodecylsulfate, magnesium aluminum silicate, and triethanolamine. It is more preferred that poloxamer 188 is used as the surface modifying agent. Oral formulations herein may utilize standard delay or time release formulations to alter the absorption of the active compound(s). Preferred oral formulations of rapamycins are

disclosed in U.S. Patents 5,559,121; 5,536,729; 5,989,591; and 5,985,325, which are hereby incorporated by reference.

In some cases it may be desirable to administer the compounds directly to the airways in the form of an aerosol.

The compounds of this invention may also be administered parenterally or intraperitoneally. Solutions or suspensions of these active compounds as a free base or pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as hydroxy-propylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof in oils. Under ordinary conditions of storage and use, these preparation contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils. Preferred parenteral formulations for administering a rapamycin are disclosed in U.S. Patents 5,530,006; 5,516,770; and 5,616,588, which are hereby incorporated by reference.

Suppository formulations may be made from traditional materials, including cocoa butter, with or without the addition of waxes to alter the suppository's melting point, and glycerin. Water soluble suppository bases, such as polyethylene glycols of various molecular weights, may also be used.

CLAIMS

1. A method of treating or inhibiting an estrogen receptor positive carcinoma in a mammal in need thereof, which comprises providing said mammal with an effective amount of a combination of a rapamycin and an antiestrogen.

2. A method according to claim 1, wherein the rapamycin is rapamycin.

3. A method according to claim 1, wherein the rapamycin is a ester, ether, oxime, hydrazone, or hydroxylamine of rapamycin

4. A method according to claim 3, wherein the rapamycin is a 42-ester or 42-ether of rapamycin.

5. A method according to claim 4, wherein the rapamycin is rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid.

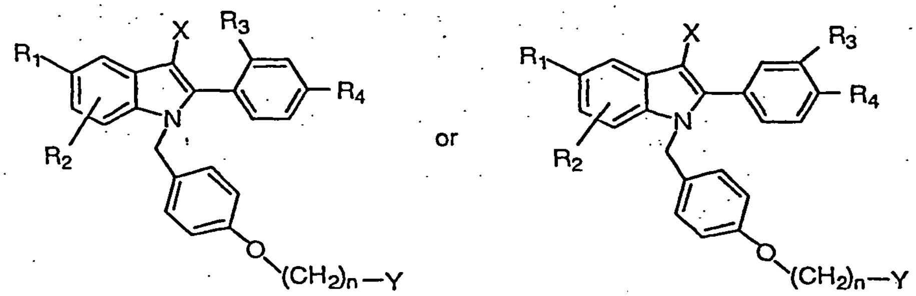
6. A method according to claim 4, wherein the rapamycin is 42-O-(2-hydroxy)ethyl rapamycin.

7. A method according to any one of claims 1 to 6, wherein the antiestrogen is tamoxifen, 4-hydroxytamoxifen, or clomiphene.

8. A method according to any one of claims 1 to 6, wherein the antiestrogen is a non-uterotrophic estrogen.

9. A method according to claim 8, wherein the non-uterotrophic antiestrogen is selected from the group consisting of raloxifene, droloxifene, idoxifine, nafoxidine, toremifene, TAT-59, levomeloxifene, LY-353381, CP-336156, MDL-103323, EM-800, and ICI-182,780.

10. A method according to claim 8, wherein the non-uterotrophic antiestrogen is a compound of formulas I or II having the structures



(I) (II)

wherein:

R_1 is H, OH, alkanoyloxy of 2-12 carbon atoms, alkoxy of 1-12 carbon atoms, halogen or mono- or poly-fluoroalkoxy of 1-12 carbon atoms;

R_2 is H, OH, alkanoyloxy of 2-12 carbon atoms, alkoxy of 1-12 carbon atoms, halogen, mono- or poly-fluoroalkoxy of 1-12 carbon atoms, cyano, alkyl of 1-6 carbon atoms, or trifluoromethyl, with the proviso that, when R_1 is H, R_2 is not OH.

R_3 and R_4 are each, independently, H, OH, carboalkoxy of 2-12 carbon atoms, alkoxy of 1-12 carbon atoms, halogen, mono- or poly-fluoroalkoxy of 1-12 carbon atoms, or cyano, with the proviso that, when R_1 is H, R_2 is not OH.

X is H, alkyl of 1-6 carbon atoms, cyano, nitro, trifluoromethyl, or halogen;

n is 2 or 3;

Y is a saturated, partially saturated or unsaturated 5-7 membered heterocycle containing a nitrogen, which may optionally contain a second heteroatom selected from the group consisting of -O-, -NH- , N(alkyl)- where the alkyl group contains 1-6 carbon atoms, -N= , and $S(O)_m$;

m is 0-2;

or a pharmaceutically acceptable salt thereof.

11. A method according to claim 10, wherein the antiestrogen is (2-(4-hydroxy-phenyl)-3-methyl-1-[4-(2-piperdin-1-yl-ethoxy)-benzyl-1H-indol-5-ol) or a pharmaceutically acceptable salt thereof.

12. A method according to claim 10, wherein the antiestrogen is (1-[4-(2-azepan-1-yl-ethoxy)-benzyl]-2-(4-hydroxy-phenyl)-3-methyl-1H-indol-5-ol) or a pharmaceutically acceptable salt thereof.

13. A method according to claim 1, wherein the rapamycin is rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid, and the antiestrogen is (2-(4-hydroxy-phenyl)-3-methyl-1-[4-(2-piperdin-1-yl-ethoxy)-benzyl-1H-indol-5-ol) or a pharmaceutically acceptable salt thereof.

14. A method according to any one of claims 1 to 13, wherein the estrogen receptor positive carcinoma is of the breast or ovary.

15. A method of treating or inhibiting estrogen receptor positive carcinoma of the breast in a mammal in need thereof, which comprises providing to said mammal an effective amount of a combination of rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid and (2-(4-hydroxy-phenyl)-3-methyl-1-[4-(2-piperdin-1-yl-ethoxy)-benzyl-1H-indol-5-ol) or a pharmaceutically acceptable salt thereof.

16. A method of treating or inhibiting estrogen receptor positive carcinoma of the ovary in a mammal in need thereof, which comprises providing to said mammal an effective amount of a combination of rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid and (2-(4-hydroxy-phenyl)-3-methyl-1-[4-(2-piperdin-1-yl-ethoxy)-benzyl-1H-indol-5-ol) or a pharmaceutically acceptable salt thereof.

17. Use of a combination of a rapamycin and an antiestrogen in the preparation of a medicament for treating or inhibiting an estrogen receptor positive carcinoma in a mammal.

18. Use according to claim 17 in which the rapamycin and antiestrogen are as defined in any one of claims 1 to 13 and 15 and 16.

19. Use according to claim 17 or claim 18 wherein the estrogen receptor positive carcinoma is of the breast or ovary.

20. A product comprising a rapamycin and an antiestrogen for administration as a combined preparation for simultaneous, separate or sequential use in treating or inhibiting an estrogen receptor positive carcinoma in a mammal.

21. A product according to claim 20 wherein the rapamycin is rapamycin.

22. A product according to claim 20 wherein the rapamycin is a ester, ether, oxime, hydrazone, or hydroxylamine of rapamycin

23. A product according to claim 22 wherein the rapamycin is a 42-ester or 42-ether of rapamycin.

24. A product according to claim 23 wherein the rapamycin is rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid.

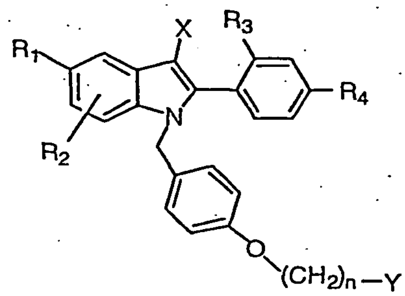
25. A product according to claim 23 wherein the rapamycin is 42-O-(2-hydroxy)ethyl rapamycin.

26. A product according to any one of claims 20 to 25 wherein the antiestrogen is tamoxifen, 4-hydroxytamoxifen, or clomiphene.

27. A product according to any one of claims 20 to 25 wherein the antiestrogen is a non-uterotrophic estrogen.

28. A product according to claim 27 wherein the non-uterotrophic antiestrogen is selected from the group consisting of raloxifene, droloxifene, idoxifine, nafoxidine, toremifene, TAT-59, levomeloxifene, LY-353381, CP-336156, MDL-103323, EM-800, and ICI-182,780.

29. A product according to claim 27 wherein the non-uterotrophic antiestrogen is a compound of formulae I or II having the structures



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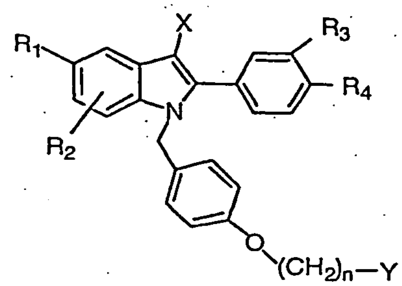

(I) (II)

wherein:

R_1 is H, OH, alkanoyloxy of 2-12 carbon atoms, alkoxy of 1-12 carbon atoms, halogen or mono- or poly-fluoroalkoxy of 1-12 carbon atoms;

R_2 is H, OH alkanoyloxy of 2-12 carbon atoms, alkoxy of 1-12 carbon atoms, halogen, mono- or poly-fluoroalkoxy of 1-12 carbon atoms, cyano, alkyl of 1-6 carbon atoms, or trifluoromethyl, with the proviso that, when R_1 is H, R_2 is not OH.

R_3 and R_4 are each, independently, H, OH, alkanoyloxy of 2-12 carbon atoms, alkoxy of 1-12 carbon atoms, halogen, mono- or poly-fluoroalkoxy of 1-12 carbon atoms, or cyano, with the proviso that, when R_1 is H, R_2 is not OH.

X is H, alkyl of 1-6 carbon atoms, cyano, nitro, trifluoromethyl, or halogen;

n is 2 or 3;

Y is a saturated, partially saturated or unsaturated 5-7 membered heterocycle containing a nitrogen, which may optionally contain a second heteroatom selected from the group consisting of -O-, -NH- , -N(alkyl)- where the alkyl group contains 1-6 carbon atoms, -N= , and $S(O)_m$;

m is 0-2;

or a pharmaceutically acceptable salt thereof.

30. A product according to claim 29 wherein the antiestrogen is (2-(4-hydroxy-phenyl)-3-methyl-1-[4-(2-piperidin-1-yl-ethoxy)benzyl-1H-indol-5-ol) or a pharmaceutically acceptable salt thereof.

31. A product according to claim 29 wherein the antiestrogen is 1-[4-(2-azepan-1-yl-ethoxy)-benzyl]-2-(4-hydroxy-phenyl)-3-methyl-1H-indol-5-ol or a pharmaceutically acceptable salt thereof.

32. A product according to claim 20 wherein the rapamycin is rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid, and the antiestrogen is (2-(4-hydroxy-phenyl)-3-methyl-1-[4-(2-piperidin-1-yl-ethoxy)-benzyl-1H-indol-5-ol) or a pharmaceutically acceptable salt thereof or 1-[4-(2-azepan-1-yl-ethoxy)-benzyl]-2-(4-hydroxy-phenyl)-3-methyl-1H-indol-5-ol or a pharmaceutically acceptable salt thereof.

33. A pharmaceutical composition for use in treating or inhibiting an estrogen receptor positive carcinoma in a mammal comprising a rapamycin and an antiestrogen or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.